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Clinical features of congenital myasthenic syndrome due to mutations in *DPAGT1*

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Abstract

Background—A newly defined congenital myasthenic syndrome (CMS) caused by *DPAGT1* mutations has recently been reported. While many other CMS-associated proteins have discrete roles localised to the neuromuscular junction, DPAGT1 is ubiquitously expressed, modifying many proteins, and as such is an unexpected cause of isolated neuromuscular involvement.

Methods—We present detailed clinical characteristics of five patients with CMS caused by *DPAGT1* mutations.

Results—Patients have prominent limb girdle weakness and minimal craniobulbar symptoms. Tubular aggregates on muscle biopsy are characteristic but may not be apparent on early biopsies. Typical myasthenic features such as pyridostigmine and 3, 4- diaminopyridine responsiveness, and decrement on repetitive nerve stimulation are present.

Conclusions—These patients mimic myopathic disorders and are likely to be under-diagnosed. The descriptions here should facilitate recognition of this disorder. In particular minimal

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craniobulbar involvement and tubular aggregates on muscle biopsy help to distinguish DPAGT1 CMS from the majority of other forms of CMS. Patients with DPAGT1 CMS share similar clinical features with patients who have CMS caused by mutations in *GFPT1*, another recently identified CMS subtype.

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Introduction

Congenital myasthenic syndromes (CMS) are a rare but increasingly recognised group of disorders characterised by fatiguable muscle weakness. They result from impaired neuromuscular junction (NMJ) transmission and to date at least 15 genes causing a CMS have been identified.1–4 Unlike autoimmune myasthenia gravis, the immune system is not involved. Molecular mechanisms of NMJ transmission failure between the subtypes are diverse and relate to the particular function of the protein at the synapse encoded by the affected gene. Some proteins have a direct role in NMJ signal transmission, such as the acetylcholine (ACh) receptor subunits or ACh esterase, while others including Rapsyn and DOK7 are involved in developing and maintaining NMJ structural integrity. The different syndromes have characteristic phenotypes and, critically, choice of treatment depends upon subtype. The more recently identified forms of CMS have a predominantly limb girdle pattern of muscle weakness that has led many to be classed as a non-specific myopathy prior to a definitive genetic diagnosis.

DPAGT1, which encodes dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosamine-phosphotransferase 1, is the most recently reported gene associated with CMS.5 This enzyme is essential for N-linked protein glycosylation, a process that starts with the assembly of the core glycan $Glc_3Man_9GlcNAc_2$ on the lipid dolichol, with the oligosaccharide being subsequently transferred from the lipid onto the asparagine residue of nascent proteins.5–7 DPAGT1 is a transmembrane ER protein that catalyses the addition of GlcNAc-1-P from cytoplasmic UDP-GlcNAc to dolichol-P, which is the first committed step of the core glycan assembly5–7 (figure 1). Mutations in *DPAGT1* are thought to affect the glycosylation of the muscle ACh receptor subunits and affect the assembly and insertion of the receptor in the post-synaptic membrane.5 *DPAGT1* has also been associated with a rare subtype of congenital disorder of glycosylation (type Ij) which has a phenotype markedly different from that of patients with CMS.8–13 Patients show benefit from anticholinesterase medication.5

Here we report in detail the clinical course of patients with CMS due to *DPAGT1* mutations in order to facilitate recognition of this disorder. We discuss the similarities and features distinguishing it from other forms of CMS and compare it to the recently identified GFPT1 CMS which may involve similar pathophysiological mechanisms.

Methods

This unexpected genetic cause of CMS was identified by an exome screen of individuals from two unrelated families who shared very similar phenotypic features. The genetics have been previously described with a brief summary of the clinical picture.5 Here, we describe

in detail the clinical phenotype of DPAGT1 CMS along with the neurophysiological and muscle biopsy findings of five patients.

Patients

All patients are the offspring of non-consanguineous parents. Patients 1, 2 and 3 are Caucasian (patient 1 and 2 originate from the UK, and patient 3 from Zimbabwe but of white European origin) and patients 4 and 5 are siblings of Argentinean descent. Patients 1–4 were assessed in the UK National Congenital Myasthenia Service in Oxford.

Consent for publication of data was obtained from all patients or their guardians participating in this study. Ethics approval for analysis of DNA and tissue samples was obtained (OXREC B: 04.OXB.017 and Oxfordshire REC C 09/H0606/74). Mutations in *DPAGT1* were detected as previously described.5

Results

Clinical features

Onset of symptoms—Gestation and early life were uneventful in all patients. The median age of onset was 2 years (range 0.5–7 years) (table 1). Patient 1 had problems weight bearing with a tendency to fall, which initially fluctuated, beginning at 2.5 years of age. Patient 2 presented with falls and difficulty keeping up with peers, and patient 3 with a delayed walking age of 2 years. Onset with hypotonia in infancy occurred in patients 4 and 5 and both had slightly late walking milestones of 18 months. Patient 4 also had poor head control.

Pattern of neuromuscular weakness at assessment—All patients had limb weakness affecting, but not restricted to, the limb girdle musculature. In patients 2 and 3, shoulder abduction weakness was marked. Distal weakness was worse than proximal in patient 1 and ankle dorsiflexion was severely affected in both patients 1 and 3. These two patients (1 and 3) were also noted to have a curled closed hand posture at rest because of weakness of thumb and finger abductors and finger extensors. Despite this marked weakness, the finger flexors of patient 1 were spared and he had a normal grip strength as measured by a dynamometer.

Eye movements were normal in all patients and only one patient (patient 4) had mild fatiguable ptosis. Two patients (2 and 5) had mild facial weakness. Two patients (1 and 2) report mild swallowing difficulties, one of whom (patient 1) also experiences intermittent slurring and nasal speech. Neck muscles were weak in four (patients 2, 3, 4 and 5) and overall flexion was more severely affected than extension.

Shortness of breath is reported by patients 1 and 2. Their forced vital capacity volumes (FVCs) are <70% and 64% predicted, respectively, although neither require respiratory support. The FVC of patients 3 and 4 was within normal range and was not tested in patient 5. Quantitative myasthenia gravis scores, where performed, ranged between 9 and 20 and are shown in table 2.

Additional features—Patient 1 has significant flexion contractures of the knees, ankles, elbows and fingers. Patients 2 and 4 have mild scoliosis, detected at age 62 and age 11, respectively. Muscle wasting is present in patient 3 only, affecting the sternocleidomastoid muscles. She also has mild pes cavus. Mild learning disability affects patients 3 and 5. Two of four patients tested (2 and 3) have non-specific abnormalities on ECG.

Progression—The longer-term course varies, although this may be age dependant.

Patient 1 was poor at sports during childhood but was able to run short distances. During childhood his walking deteriorated and he started to use a wheelchair at around 9 years of age. At that time he had a muscle biopsy and was told he had Werdnig-Hoffman spinal muscular atrophy. It became apparent over subsequent years that this diagnosis was not correct and CMS was suspected. In adult life he has been stable but with long-term fluctuations lasting weeks to months not associated with exacerbating factors such as infection.

Patients 2 and 3, who are now in their sixth decade, report slowly progressive weakness. Patient 2 worsened during adolescence and began to have frequent falls and was noted to waddle when she walked. Around this time she developed difficulty lifting objects above her head and playing netball. Her condition stabilised in her 20s, although she had a prolonged apnoea following suxamethonium administration during an operation at age 22. Her weakness later progressed and she developed truncal weakness at age 30. She reports increasing neck weakness in her fifth and sixth decades. Patient 3, who is most severely affected, reports worsening around age 7. She was diagnosed with autoimmune myasthenia gravis at that time and underwent thymectomy with no benefit. She continued to deteriorate, and by age 14 was using a wheelchair most of the time. In her 40s she had plasma exchange with no effect. During adult life her weakness has worsened and while at 49 she was able to walk 20 paces, now at age 58 she transfers independently but is unable to take steps.

The siblings, patients 4 and 5, who are the youngest, report some improvement in their teens and childhood, respectively. Following improvement in his teens, patient 4 was able to walk 5 km without support. He can also climb stairs and run, although his running style is abnormal and slower than his peers. Patient 5 has generalised hypotonia although she can walk, run and jump.

Response to treatment—All five patients respond to pyridostigmine, although patient 5 (aged 6 years) does not take any medication because currently she is only mildly affected. The response was dramatic in patients 1 and 2 but modest in patient 3. Patient 1 recalls being able to run along a corridor and lifting items above his head after being given pyridostigmine initially as a child. Patient 3 describes feeling like ‘Wonder Woman’ after initial pyridostigmine, although she describes the effect of this diminishing over years of treatment. Two of three patients benefited from 3, 4-diaminopyridine (patients 1 and 3), but the third was unable to tolerate the drug due to side effects (patient 2). Oral salbutamol treatment resulted in a dramatic improvement in patient 1 when started at age 42; this prevented his major fluctuations, improved his grip and has enabled him to go from being wheelchair bound to walking within his home. He has noted cramps secondary to this treatment.

Investigations

Biochemical studies of glycosylation—Transferrin (Tf) glycoform analysis is used to detect disordered glycosylation and can be used in the evaluation of congenital disorders of glycosylation (CDG) where they help to classify CDG syndromes (table 2). There are a number of different laboratory methods and in the patients described here, either Tf isoelectric focusing (IEF) or determination of percentage carbohydrate deficient Tf (% CDT) by immunoturbidimetry was performed.14 Both methods determine the degree of glycosylation of the serum glycoprotein Tf. Under normal circumstances, serum Tf separates into different isoforms depending on degrees of hypoglycosylation, though tetrasialo-Tf (which has four sialic acid residues) is the most abundant form. Aberrant glycosylation causes an increase in hyposialated isoforms. Sialic acid residues are negatively charged and Tf IEF separates the differently charged Tf isoforms into bands on an agarose gel using electrophoresis. The pattern of these bands can help to distinguish between type I and type II CDG. In measuring % CDT by immunoturbidimetry, the hyposialated (carbohydrate deficient) isoforms are quantified and the sum of these relative to total Tf is expressed as a percentage. While the latter is reportedly less sensitive and less specific than IEF, it is less labour intensive.14 Results of studies performed are shown in table 2.

Neurophysiology—All patients had findings consistent with abnormal NMJ transmission on electrophysiological testing (table 2). Detailed results of repetitive nerve stimulation were available in three patients (patients 1, 3 and 4) and showed greater proximal abnormality. Extra discharges to single stimuli were seen in the hypothenar muscles of patient 3 during a motor nerve conduction study at age 49 but not at age 45 years and were presumed to be a treatment effect. Repetitive discharges were not reported in other patients.

Muscle biopsy—Patients 1–4 underwent muscle biopsy and tubular aggregates (TA) were demonstrated in all in at least one biopsy (table 2). The third biopsy from patient 2 was from an intercostal muscle and detailed study of the NMJ indicated a post-synaptic defect. Endplates were of normal size and had a normal amount of acetylcholinesterase activity but showed reduced α-bungarotoxin binding at 11.2 amol/endplate (normal range 13–30), indicative of receptor deficiency. Electrophysiological studies demonstrated a reduction in miniature endplate potential amplitude to less than 50% of normal, also in keeping with receptor deficiency.

In the biopsy from patient 3 at age 47, small TA in type II fibres were visualised best in the nicotinamide adenine dinucleotide dehydrogenase tetrazolium reductase (NADH-TR) preparation. In addition, a number of vacuolated fibres containing material, most of which had staining characteristics of glycogen (positive in the periodic acid Schiff preparation and digested by prior treatment with diastase), while in a few vacuoles this material was resistant to diastase, suggesting accumulation of another polysaccharide. Ultrastructural examination confirmed the presence of TA, an excess of glycogen and autophagic vacuoles. Repeat biopsy at age 57 showed similar appearances.

Patient 4 had two muscle biopsies performed at the ages of 5 and 9 years and although ultrastructural abnormalities were observed in the first biopsy, these were not identified as

TA. The second biopsy specimen showed inclusions in type II muscle fibres, with light microscopy features of TA.

Discussion

We describe the clinical and investigative features of a newly identified subtype of CMS caused by mutations in *DPAGT1,* a gene involved in a ubiquitous glycosylation pathway. As is typical of CMS, these patients have an autosomal recessive pattern of inheritance and present with muscle weakness early on in life which responds to anticholinesterase medication. Features that may be more specific are a delayed onset beyond infancy, a limb girdle pattern of weakness, sparing of the ocular and facial muscles and a myopathy associated with TA. Although DOK7 CMS presents at a similar age of onset, causes a limb girdle pattern of weakness and spares extra-ocular muscles, it does not respond to pyridostigmine and TA are not seen on muscle biopsy. Other forms of CMS can be distinguished by eye movement impairment (mutations in the AChR genes), early life crises (Rapsyn and ChAT CMS and fast channel syndrome) and worsening with pyridostigmine (slow channel syndrome and *COLQ* mutations). DPAGT1 CMS appears similar to the recently described GFPT1 CMS,15 16 where the affected enzyme catalyses a step in the hexosamine synthetic pathway that generates products that can feed into the N-linked glycosylation pathway.

DPAGT1 is involved in the first steps of the core glycan assembly before it is transferred onto the asparagine residue of nascent proteins—N-linked glycosylation.6 7 The muscle acetylcholine receptor (AChR) is a glycoprotein with all subunits known to undergo N-linked glycosylation. It is proposed that mutations in *DPAGT1* impair AChR subunit glycosylation leading to reduced assembly and transport of the AChR into the postsynaptic membrane, and thus reduced endplate AChR number.5 This is likely to be the major mechanism for impaired neuromuscular transmission, although impaired glycosylation of other proteins located at the NMJ may also contribute to the disorder. GFPT1 is the key enzyme in the biosynthetic pathway that provides amino sugars for the synthesis of glycoproteins, glycolipids and proteoglycans.5 Although the underlying mechanism for how mutations in *GFPT1* result in a myasthenic syndrome is not fully elucidated, the similarity of clinical features shared between patients with *GFPT1* mutations and *DPAGT1* mutations suggests that the same N-linked glycosylation pathway is involved. GFPT1 CMS also has a later age of onset, spares eye movements and has only minimal facial and bulbar involvement. Ptosis is also either mild or absent. Limb girdle weakness is prominent in both DPAGT1 and GFPT1, although it is not uncommon for either subtype to have additional distal weakness. In particular, two DPAGT1 individuals had marked hand weakness causing the hands to be curled when closed.

DPAGT1 and GFPT1 CMS are both pyridostigmine responsive, although in our experience a 3, 4-diaminopyridine add-on is beneficial. One DPAGT1 patient has been treated with oral salbutamol and this resulted in a dramatic functional improvement. Oral salbutamol and ephedrine are used effectively in some other forms of CMS including CMS due to mutations in *DOK7*17–20 and *COLQ*17 21 and are thought to exert their effect by stimulating muscle β2 adrenergic receptors. This may help maintain post-synaptic structure, thereby conserving

the safety factor. Of the two GFPT1 patients treated by the CMS Service in Oxford, one has tried salbutamol and its effect has been modest. Ephedrine has been effective in the second and could be an alternative in DPAGT1 CMS where salbutamol is not tolerated or effective.

These two conditions may be misdiagnosed as myopathy, although an elevated serum creatinine kinase (CK) level was not found in any of the DPAGT1 patients. This is in contrast to GFPT1 CMS where CK levels are commonly elevated (50% of patients studied).16 The presence of TA on muscle biopsy in DPAGT1 and GFPT1 is a useful feature distinguishing them from other forms of CMS, although these were not seen in early biopsies from our DPAGT1 patients, suggesting they accumulate over time. TA have been observed in some cases of myotonia, myotonic dystrophy and channelopathies. Additionally, a poorly defined 'tubular aggregate myopathy' is described22–25 associated with myalgia and cramps. It is possible that this group represent a number of different disorders. TA visualised by electron microscopy are inclusions of tubules of variable appearance within muscle fibres.26 It is generally accepted that these structures derive from the sarcoplasmic reticulum (SR), although the mechanism of their formation has not been elucidated. It has been suggested that reshaping of the SR and TA formation is initiated by aggregation of misfolded proteins.22 Where glycan residues have a critical contribution to the structural integrity of a protein, it follows that disordered glycosylation due to mutations in genes such as *DPAGT1* and *GFPT1* could lead to protein misfolding and predispose to TA formation.

Previously, mutations in *DPAGT1* have been reported in connection with congenital disorder of glycosylation type Ij. CDG are a group of disorders caused by defects in the formation (type I) or processing (type II) of glycoproteins or glycolipids. The majority are recessive and often present with multisystemic disease. Great variability in clinical features is reported.10 Type Ij is an infrequent CDG with just 11 individuals (seven families) with an identified genetic mutation reported found in the literature.8–13 27 In nine of these patients, clinical details have been reported (table 3) and clear multisystem involvement is described in the majority. In six of the nine, symptom onset was within the first day of life, with death occurring before 3 years of age in five. Some clinical features are shared, with six having had seizures (three refractory), seven had hypotonia and five had microcephaly. However, there is significant variation in clinical features and severity, with two siblings (patients x and xi), aged 34 and 32 years at the time of the report,13 presenting with a relatively mild phenotype. A severe fetal hypokinesia phenotype was described in patient ix11 with no spontaneous movements and very little movements with stimuli. Absence of intra-uterine movements was reported by his mother and he had joint contractures. Fetal hypokinesia and contractures are associated with some forms of CMS (particularly Rapsyn CMS and mutation of the AChR γ subunit). Patients iv and v were from a large consanguineous family reported to have two siblings and 14 cousins who also died in infancy. Although these family members were not genetically tested for the *DPAGT1* mutation, they were thought to be affected.

None of the mutations in the DPAGT1 CMS patients were shared by the CDG-Ij cases. All DPAGT1 CMS patients reported are compound heterozygotes for missense mutations with the exception of patient 2 who carries the duplication mutation c.699dup which results in a premature stop codon in addition to a missense mutation. All DPAGT1 CMS patients had a

missense mutation in exon 3 and it has been suggested that this exon may be especially important for a function related to neuromuscular transmission.5 However, two siblings with CDG-Ij reported to have severe multisystem disease were both homozygous for the exon 3 mutation c.341C>G.9 In the CDG-Ij cases with hypotonia, details of whether single-fibre electromyography or repetitive nerve stimulation was performed are not reported. Therefore, it is not known whether there was any defect of neuromuscular transmission, although this remains a possibility. At present the numbers of cases with *DPAGT1* mutation are too small to make genotype–phenotype correlations.

Why the patients with mutations that we have identified have symptoms largely restricted to a NMJ disorder is not understood, although there are a number of possibilities. Different mutations may result in different levels of functional DPAGT1 enzymatic activity and it is possible that those with disease restricted to the NMJ have higher levels of enzyme function and thus a milder phenotype than those with multisystem disease. Alternatively, it may be that appropriate glycosylation of AChR (or other NMJ proteins) is more critical to normal function than in proteins of other tissues, thus making the NMJ more susceptible to a modest impairment in DPAGT1 function. A further possibility is that DPAGT1 has an uncharacterised muscle-specific function and these patients' mutations specifically disrupt that function while the protein can function adequately in other cell types.5 Two of the cases reported here have mild learning disability. Cognitive impairment is not a typical feature of congenital myasthenia and may be incidental in these cases. Cranial MRI was performed in only one of these cases (patient 5) and was normal.

Biochemical tests may help to identify patients with DPAGT1 CMS. However, the sera of two patients (patients 2 and 3) tested by IEF were normal. This would be consistent with the hypothesis that the reduction in DPAGT1 enzymatic activity is less marked in these patients than in those with CDG type Ij, where hypoglycosylation of serum Tf was demonstrated in all six families with clinical details reported.8–13 The sera of patients 4 and 5 were tested by another method (immunoturbidimetry)28 and were abnormal, confirming at least some generalised abnormality of glycosylation. Thus in patients with a limb girdle phenotype and decrement, testing for N-linked glycosylation defects should be considered and may bypass the need for more invasive investigation.

In summary, this report details the clinical features of a novel CMS, referred to here as DPAGT1 CMS. This newly defined CMS shares many clinical and histological features with GFPT1 CMS. The pathophysiological mechanism of GFPT1 CMS has not been defined, but the similarity in clinical features suggests a similar molecular pathology. Both the underlying pathophysiology of abnormal protein glycosylation and the described phenotype set DPAGT1 CMS and GFPT1 CMS apart from the majority of other CMS subtypes. In particular, the minimal craniobulbar involvement, with absence of both ophthalmoparesis and ptosis, is characteristic of both DPAGT1 CMS and GFPT1 CMS and can help to distinguish them from other CMS subtypes. In a patient with CMS, TA on muscle biopsy is highly suggestive of a mutation in a glycosylation pathway gene. Their presence should direct genetic testing towards *DPAGT1* and *GFPT1* in preference to previously reported CMS associated genes. Raised CK levels may help to distinguish between GFPT1 CMS and

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DPAGT1 CMS. Although the number of DPAGT1 cases reported here is small, the CK level was normal in all, while it is commonly elevated in GFPT1 CMS.

It is likely there are patients with DPAGT1 CMS who currently carry a diagnosis of an undefined myopathic disorder and may be under the care of another specialist. A defined genetic diagnosis will be informative for appropriate treatment. Moreover, given the extensive metabolic pathways involved in glycosylation, it is likely that further genes associated with CMS will lie within these pathways.

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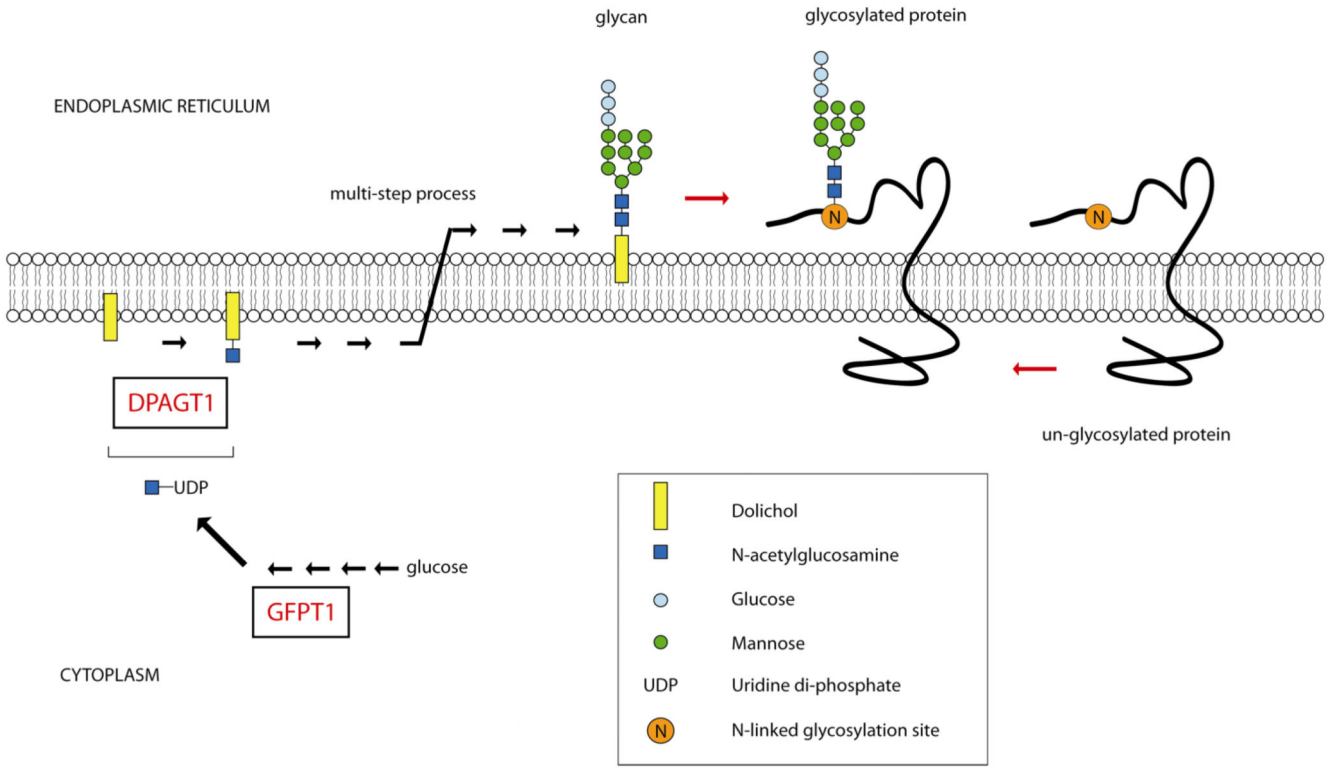

Figure 1.

N-linked glycosylation pathway. GFPT1, glutamine-fructose-6-phosphate transaminase 1; DPAGT1, dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminephosphotransferase 1. Cytoplasmic glucose is the initial substrate in the hexosamine biosynthetic pathway which comprises multiple reactions catalysed by different enzymes of which GFPT1 is key. A major product of this pathway is UDP-GlcNAc (or UDP-N-acetylglucosamine), an essential substrate for N-linked protein glycosylation. DPAGT1 catalyses the first committed step of N-linked glycosylation in which phosphorylated UDP-GlcNAc from cytoplasmic UDP-GlcNAc is added to a transmembrane lipid carrier, dolichol phosphate. The core glycan is assembled by successive addition of individual sugar residues (mannose and glucose) with each step catalysed by a different enzyme. During assembly the oligosaccharide is transported across the membrane to the endoplasmic reticulum interior where further sugar residues are added. Once fully assembled, the core glycan is released from dolichol ready to be transferred to an N-linked glycosylation site on nascent protein.

Table 1

Clinical features

		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Kinship		1	2	3	4	4
Mutations		c.324G>C (p.Met108Ile) and c.349G>A (p.Val117Ile)	c.349G>A (p.Val117Ile) and c.699dup (p.Thr234Hisfs*116)	c.478G>A (p.Gly160Ser) and c.574G>A (p.Gly192Ser)	c.358C>A (p.Leu120Met) and c.791T>G (p.Val264Gly)	c.358C>A (p.Leu120Met) and c.791T>G (p.Val264Gly)
Gender		M	F	F	M	F
Age (years)						
Onset		2.5	7	2	0.5	First year
Assessed		43	57	58	17	6
Current		43	57	58	25	6
Ptosis/ophthalmoplegia		No/no	Mild/no	No/no	No/no	No/no
Facial/bulbar weakness		No/mild	Mild/mild	No/no	No/no	Mild/no
Neck weakness		No	Flexion only	Flexion only	Flexion only	Yes
Proximal weakness	UL	+	+++	+++	++	++
	LL	+	+	+++	++	+
Distal weakness	UL	+++	++	++	++	+
	LL	+++	+	+++	++	+
QMG score		9/39	18/39	20/39	ND	ND

LL, lower limb; ND, not done; QMG, quantitative myasthenia gravis (a higher score indicates greater weakness); UL, upper limb; +, mild; ++, moderate; +++, severe.

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Table 2

Investigations

			Electrophysiology			Muscle biopsy
Patient	CK	Biochemical tests of glycosylation	RNS % decrement/muscle	SFEMG abnormal/ % potentials with raised jitter or block	EMG myopathic	Muscle/age (years)/tubular aggregates detected
1	Normal	ND	38%/deltoid, 21%/ADM	+/77%	ND	1. Deltoid/9/− 2. Quadriceps/19/+
2	Normal	Transferrin IEF normal	Abnormal (value unknown)	+/Unknown	+	1. Deltoid/16/− 2. Wrist extensor/ 20/+ 3. Intercostal muscle/32/not tested
3	Normal	Transferrin IEF normal	33%/anconeus, 19%/ADM	+/93−100%	+	1. Deltoid/47/+ 2. Deltoid/57/ aggregates which resemble TAs
4	Normal	% CDT by immunoturbidimetry raised (8.5%)	30%/anconeus, no decrement/ADM	+/84%	+	1. Unknown/5/ ultrastructural abnormalities detected although not identified as TAs 2. Unknown/9/+
5	Normal	% CDT by immunoturbidimetry raised (5.98%)	Abnormal (value unknown)	ND	ND	ND

ADM, abductor digiti minimi; CK, creatinine kinase; CDT, carbohydrate deficient transferrin (normal range 1.39–2.65%); EMG, electromyography; IEF, isoelectric focussing (see text for explanation of this biochemical test of glycosylation); ND, not done; TA, tubular aggregates; RNS, repetitive nerve stimulation; SFEMG, single-fibre electromyography.

Table 3
Reported cases of congenital disorders of glycosylation type Ij

Patient	Reference and year reported	Mutations	Sex/onset age/onset symptoms/age at death	Clinical features
i	Wu *et al*, 20038	c.660A>G (p.Tyr170Cys) and unspecified splicing defect	F/4 months/infantile spasms/alive at 6 years	Developmental delay, microcephaly, arched palate, micrognathia, exotropia, fifth finger clinodactyly, single flexion creases, skin dimples on upper thighs, severe hypotonia, refractory seizures
ii	Vuillaumier-Barrot *et al*, 200527	c.890A>T (p.Ile297Phe) and c.162–8G>A (splicing defect)	Not reported	Not reported
iii			Not reported	Not reported
iv	Imtiaz *et al*, 201212	Homozygous c.902G>A (p.Arg301His)	M/birth/respiratory distress/5 years	Developmental delay, hypotonia, microcephaly, cyanotic spells, elevated ALT, elevated CK, delayed myelination on brain MRI, seizures, muscle biopsy showed fibre type disproportion
v			M/birth/hypotonia, poor feeding, choking/7 months	Decreased fetal movement, microcephaly, elevated ALT
vi	Würde *et al*, 20129	Homozygous c.341C>G (p.Ala114Gly)	F/day 1/hyperexcitability/8 months	Developmental delay, reduced spontaneous movement, hypocalcaemia, hypotonia, refractory seizures, bilateral cataracts, mild hepatomegaly, inverted mammillae, nystagmus, strabism, hypertrichosis, progressive microcephaly, cerebral atrophy, long eyelashes, slight elevation of liver enzymes
vii			F/day 1/tremor, fasciculations, abnormal movements/1 year	Developmental delay, reduced spontaneous movement, hyperreflexia, pathological fat pads, shortened Achilles tendons, bilateral cataracts, refractory seizures, progressive microcephaly, strabism, nystagmus, slight elevation of liver enzymes
viii	Timal *et al*, 201210	c.206T>A (p.Ile69Asn) and c.161+5G>A (splicing defect)	M/birth/asphyxia/2.5 years	Respiratory insufficiency, apnoeas, jaundice, elevated liver enzymes, bilateral nuclear cataract, cryptorchidism, dysmorphia, hypertonia of extremities, joint contractures, tremor, feeding difficulties
ix	Carrera *et al*, 201211	c.901C>T (p.Arg301Cys) and c.1054T>G (p.Leu385Arg)	M/birth/abnormal fetal monitoring—emergency caesarean section, mechanical ventilation/1.5 months	No intra-uterine movements felt, fetal hypokinesia phenotype, unexpressive face without nasolobial grooves, soft

Patient	Reference and year reported	Mutations	Sex/onset age/onset symptoms/age at death	Clinical features
				long ears, U-shaped vermillion of upper lip, thick skin, hypertrichosis, hand camptodactyly, moderate multiple contractures, hypotonia, severe hypokinesia
x	Iqbal *et al*, 201213	c.85A>T (p.Ile29Phe) and c.503T>C (p.Leu168Pro)	F/5 years/epilepsy, hypotonia, aggressive behaviour/alive at 34 years	Mild atypical facial dysmorphism, night blindness, moderate IQ (35–49), abnormal speech, balance problem
xi			M/2 years/epilepsy, hypotonia, aggressive behaviour/alive at 32 years	Mild atypical facial dysmorphism, moderate IQ (35–49), abnormal speech, balance problem

ALT, alanine aminotransferase; CK, creatinine kinase; IQ, intelligence quotient.

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